SPIROCHAETA RECURRENTIS: A FILTER PASSER

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Spirochaetes in an infective form can be forced through a Berkefeld filter by pressures of 50 pounds, and over, to the square inch (Todd and Wolbach, 1914). This note records an endeavor to ascertain the form in which *Spirochaeta recurrentis* passes through the filter.

Wolbach (1915) has shown, by sections, that *Spirochaeta elusa* is present everywhere in the walls of a Berkefeld filter through which these organisms have passed. Nine attempts were made to see *Spirochaeta recurrentis* in Berkefeld filtrates. Since all the experiments were of the same character, the description of one describes all.

(Experiment 595). Two, or more, rats were chloroformed. Heart blood was pipetted off. Organs were ground up with sharp sand in a 1 per cent. solution of sodium citrate in normal saline. The blood, with enough citrate solution to prevent coagulation and the ground-up organs, was passed through a well-impacted Buchner filter in order to remove red cells and organ débris. To prove the presence of spirochaetes in the Buchner filtrate specimens were examined and, to prove the infectivity of the spirochaetes seen, rats were inoculated. The growth of *Bacillus prodigiosus* from a four-day-old culture was then washed into 5 ccm. of normal saline and added to the Buchner filtrate. Control tubes, in which *B. prodigiosus* grew invariably, were inoculated from the filtrate. The Buchner filtrate was then passed through a Berkefeld filter under pressure varying from 50 to 90 pounds. To prove that the filters were intact culture tubes were inoculated with the filtrate; *B. prodigiosus* grew in none. To prove the infectivity of the Berkefeld filtrate rats were inoculated with it. To ascertain the form in which spirochetes existed in the filtrate it was examined.

In seven experiments, rats inoculated with Berkefeld filtrate became infected (Todd and Wolbach, 1914); in only two, of these seven, experiments were spirochaetes seen in the Berkefeld filtrate. In all nine experiments, infective material came from rats which were at the height of a first attack by one of four strains of *Spirochaeta recurrentis*. Each of the strains was known to produce a marked infection in white rats. The filters employed were either "W" or "N" Berkefeld filters. "W" filters were used for both experiments in which spirochaetes were seen in the filtrate.

There are two methods of proving the presence of spirochaetes. The first is the inoculation and demonstrated infection of a susceptible animal. The second is the detection of the parasites by microscopical examination. Both methods are fallacious. Each may reveal an infection where the other fails to do so.

The inoculation of susceptible animals is not an infallible test for the presence of spirochaetes. In these experiments, the strains

employed usually produced heavy infection in white rats, with many spirochaetes in the blood; young white rats were used since they are more susceptible to infection than are older ones. Yet, a few rats, shown to be susceptible by subsequent re-inoculation with the same strain, resisted inoculation by material in which spirochaetes were shown to be present and infective. Spirochaetes were present in resistant rats not at all or in numbers too small to be detected by the microscopical examination of blood. In some instances, spirochaetes have been shown to be present in a resistant rat by aspirating, under chloroform, blood from its heart and by inoculating and infecting with it a fresh rat.

Even repeated microscopical examination may fail to reveal spirochaetes in material known to contain them. As a rule, blood is examined for spirochaetes in thick films, dehemoglobinized and stained by some modification of Romanowsky's method. In order to compare this method with the examination of fresh preparations by dark ground illumination, a series of sixty-nine observations was made in which blood that might easily contain spirochaetes was examined simultaneously by the thick film and dark stage methods. There is little to chose between them. Twenty-six examinations were positive by the dark stage method and twenty-five by the thick film; four times spirochaetes were detected by the dark stage method alone, and thrice spirochaetes were found in thick films when they were unseen by dark stage examination. Thin preparations of blood, covered with ¾-inch square coverslips and ringed with vaseline were used for the dark stage examinations. They were always examined soon after they were made. Ten minutes were spent in the examination of each specimen, whether stained or fresh, before a negative examination was recorded.

Spirochaetes are not thrown down by centrifugalization as are trypanosomes (Dutton and Todd, 1905). Yet, spirochaetes may be seen in films of the precipitate thrown down by centrifugalization of fluids (coxal fluid from ticks, blood) in which previous examination failed to reveal them. Spirochaetes were found in precipitates obtained by centrifugalization at slow speeds, 200 to 500 revolutions per minute, for twenty minutes as well as in fluids centrifugalized at higher speeds, 2,000 to 3,000, for long periods, 90 to 240 minutes. Centrifugalization at low speeds was done in a small centrifuge, distance from the center to the bottom of the tubes being 14 cm. Centrifugalization at high speeds was done in a large centrifuge, the distance from the center to the bottom of the tubes being 24 cm. Ordinary urine centrifuge tubes holding 10 c.cm. were usually employed. It is of advantage to centrifugalize in two stages. Fluid is taken from the bottom of the

tubes first centrifugalized and placed for the second centrifugalization in smaller tubes each about 6 mm. in diameter and 13 cm. in length.

In order to ascertain whether all infective spirochaetes can be brought to the bottom of centrifuge tubes by centrifugalization, infective material was centrifugalized at high speeds, from 1,400 to 3,500 revolutions per minute, for periods ranging from twenty minutes to four hours. The top one-fifth or one-third of the fiuid was drawn off with a syringe, so soon as the centrifuge stopped, and injected into rats. In six out of nine experiments rats so inoculated became infected. In seven of these experiments the precipitate was searched for spirochaetes; they were found in three instances. Twice, the spirochaetes seen in the precipitate from fluids centrifugalized at high speeds were broken.

There was no conspicuous peculiarity in the morphology of the spirochaetes twice found in precipitate from centrifugalized Berkefeld filtrates. There were a few small forms, which would have been seen with difficulty in fresh preparations without dark field illumination; but, on the whole, the parasites were, if anything, rather larger than usual.

SUMMARY

1. *Spirochaeta recurrentis* can be forced in its type form through a "W" Berkefeld filter.

2. Centrifugalization, at the speeds and for the times employed, does not throw down all infective forms of *Spirochaeta recurrentis*.

REFERENCES

Todd, J. L., and Wolbach, S. B. 1914.—Concerning the Filterability of *Spirochaeta duttoni*. Jour. Med. Research, 30: 27-36.

Dutton, J. E., and Todd, J. L. 1905.—The Nature of Human Tick Fever in the Eastern Part of the Congo Free State. Liverpool School Trop. Med., Mem. 17.

Wolbach, S. B. 1915.—On the Filterability and Biology of Spirochaetes. American Jour. Trop. Dis. Prev. Med., 2: 494-505.